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Coating of commercially available materials with a new heparinizable material

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Different commercial materials, such as polyurethane (PU), plasticized PVC (PVC), glass, Gore-tex, and Dacron, were coated with a well-characterized biomaterial (PUPA) based on polyurethane and poly(amido-amine) components. Two different classes of coating were obtained due to the different characteristics of the substrates. In the case of PVC and polyurethane which are soluble in the solvent of the PUPA-coating solution, there was

penetration and blending of the coating and underlying materials. In the case of glass, Gore-tex, and Dacron, which are insoluble in the solvent of the coating solution, only a superficial layer of PUPA could be obtained. The coating stability was investigated and the interaction between coating and underlying material studied by FT-IR. All the stable coatings showed the ability to bind as much heparin as PUPA material by itself.

INTRODUCTION

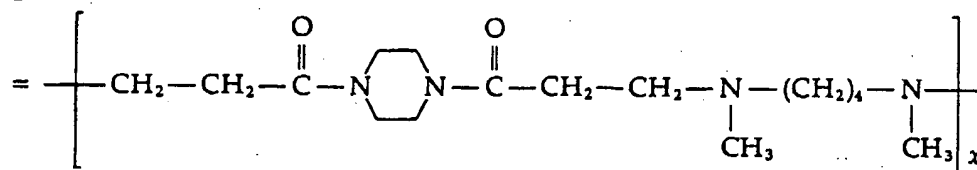
The challenge of biomaterial research lies in fabricating materials that maintain mechanical integrity and biocompatibility in contact with blood. The utility of these devices is complicated by thrombosis. It is generally accepted that thrombotic events are stimulated by and occur directly at the surface of the material through the initiation of the intrinsic clotting cascade and the adhesion/aggregation of activated platelets.¹ The nature of thrombotic events demands that these two synergistic factors be controlled if the biocompatibility of the surface is to be optimized.

Current research is concerned with investigating surface modifications to improve the "performance" of these devices. Of the different approaches for optimizing surface biocompatibility, surface heparinization is a well accepted strategy. Heparinized materials appear to be highly compatible with both platelets and the plasma coagulation system.²

Our starting point in fabricating materials with improved biocompatibility is the use of poly(amido-amine)s which have been shown in previous studies to have the capacity to form a stable complex with heparin.³ Poly(amido-amine)s maintain their heparin-binding ability even if present as a component in a given material. N₂LL poly(amido-amine), obtained by polyaddition

of *N,N'*-dimethyl-1,4-diamino butane to 1,4-bis(acryloyl)piperazine,

N_2LL



was recently cross-linked with commercial polyurethane (PU) via hexamethylenediisocyanate (HMDI). The properties of the material (PUPA), obtained as a film by evaporating the solution containing the three components in a given ratio in *N,N'*-dimethylformamide (DMF), which is capable of strongly adsorbing high quantities of heparin, have already been discussed.⁴ Instead of preparing new materials, the PUPA solution may be used to coat commercially available materials.

The aim of the present study was to provide a blood-compatible, heparinizable coating to improve the surface properties of existing polymer substrates without changing the bulk properties. This paper presents the initial results of preparation and surface characterization of different commercial materials [plasticized polyvinylchloride (PVC), polyurethane (PU), glass, polytetrafluoroethylene (Gore-tex), and polyethyleneterephthalate (Dacron)] coated with PUPA and their heparin-adsorbing properties.

MATERIALS AND METHODS

Materials

Pellets of the commercial polyurethane Pellethane R2363-80AE were purchased from Upjohn Polymers. The pellets were purified by extraction in Soxhlet with $\text{CH}_3\text{OCH}_3/\text{CH}_3\text{OH}$ (v/v; 1/2.5) over a period of 3 days.

Tubes of medical-grade plasticized PVC were purchased from Baxter.

Dacron in the form of medical polyethyleneterephthalate patch fabrics was purchased from Sorin Biomedica S.p.A.

Gore-tex in the form of polytetrafluoroethylene membranes supported on polypropylene fabric (0.200 μm thick) was purchased from W.L. Gore & Ass. S.r.L.

Glass in the form of Deckglasser coverslips was purchased from Perfect.

Films of polyurethane

These were obtained by evaporation from a 10% (v/v) solution of the polyurethane pellets in *N,N'*-dimethylformamide (DMF) at 70°C *in vacuo* (400 mbar).

Films of PVC

These were obtained by slow evaporation from a 10% (v/v) solution of the medical-grade plasticized PVC tubes in tetrahydrofuran (THF) at room temperature. The film obtained showed the same IR spectrum as commercial PVC.

PUPA solution

The synthesis of PUPA involves two reaction steps, as previously described⁴:

(1) Poly(amido-amine) with terminal free isocyanate groups were obtained by adding a large excess of hexamethylenediisocyanate to the dry and alcohol free methylene chloride solution of the amino end-capped poly(amido-amine) N₂LL under nitrogen.

(2) 10% (v/v) of Pellethane^R 2363-80AE in *N,N'*-dimethylformamide (DMF) was added to the above methylene chloride solution under nitrogen and vigorous stirring. The mixture was kept at 60°C under nitrogen and vigorous stirring for 3 h. After this time it could be used to coat the different substrates.

Coating procedure

(1) Polyurethane films, PVC films and glass plates were coated by pouring the PUPA solution in DMF at 60°C over the different materials under a nitrogen stream, in a sufficient quantity to cover the entire substrate surface. Evaporation of the solvent (DMF) was performed at 60°C *in vacuo* (400 mbar).

(2) Dacron and Gore-tex fabrics were bathed in the PUPA solution at 60°C under magnetic stirring for 5 h (without any further treatment) and then rinsed with DMF. Both these steps were carried out under a nitrogen stream. The DMF was then evaporated off at 60°C *in vacuo* (400 mbar). PUPA solutions in DMF, at three different concentrations (80%, 50%, and 30% PUPA by volume) were used to coat the Dacron fabrics.

Slices 40 or 50 μ m thick of the PUPA-coated PU and PVC samples were obtained by a microtome (Sartorius).

Extraction

(1) Samples of PUPA-coated polyurethane, Dacron, and Gore-tex were extracted in Soxhlet with CH₃OH/H₂O (6:1; v/v) for 5 h.

(2) Samples of PUPA-coated PVC were extracted in Soxhlet with pure water for 5 h.

(3) Samples of PUPA-coated glass were simply washed with CH₂Cl₂.

Stability of PUPA coating

Films of PU and PVC and Dacron and Gore-tex fabrics coated with PUPA were flexed and elongated.

Cracks did not appear for PU, PVC films, and Dacron fabrics. They only appeared for Gore-tex if it was strongly flexed. The stability of the layer of PUPA on Dacron and Gore-tex fabrics was investigated as follows: the samples, once weighed, were placed in a 7-mm-i.d. Silastic (tetramethylsiloxane) tubing circuit connected with a saline solution reservoir, and the apparatus was thermostatted at 37°C. A peristaltic pump (rate = 25 rpm; flow = 100 mL/min) was used to perfuse the circuit with saline solution. The experiments were stopped after 1 month. The samples were then removed, rinsed with 0.01 M NaOH solution, washed with water, dried *in vacuo* (400 mbar), and weighed.

The layer of PUPA was removed from glass plates by scratching when the sample was put in contact with water, CH_2Cl_2 , and DMF.

SURFACE CHARACTERIZATION

Optical microscopy

Surfaces of PUPA-coated and native Dacron samples were analyzed by inverted optical microscope (Nikon: Diascopic Die Nomarski attachment TMD-NT). Photomicrographs were taken of representative areas of the surface.

FT-IR/ATR spectroscopy

ATR spectra of the samples were recorded on a Perkin-Elmer FT-IR M 1800 between 4000 and 750 cm^{-1} . An MCT detector was used and the apparatus was purged with nitrogen. Typically 300 scans at a resolution of 2.0 cm^{-1} were averaged and the spectra were stored on a magnetic disc. The frequency scale was internally calibrated with a reference helium-neon laser to an accuracy of 0.01 cm^{-1} . A KRS-5 crystal was used at an angle of 45°.

Spectral subtraction

When taking the spectrum of a coated material, the beam sees the coating layer and the underlying material for the penetration depth of the beam into the sample. It is therefore necessary to subtract the spectrum of the underlying material in order to analyze any changes in the spectrum of the coating, or vice versa.

The spectra of the native and coated substrate were taken at first. Subtraction was then carried out according to the null criterion, by suppressing appropriate bands to baseline level. In order to remove the characteristic bands of the substrate, a particular band near bands of interest is selected and nulled. This should lead to the removal of the other substrate bands, however, some negative and/or additional positive peaks of the substrate may appear in the difference spectrum due to the spectral differences between the spectrum of the native substrate and that of the substrate which interacts with the coating material.

Titration of the aminic nitrogen atoms

The determination of the poly(amido-amine) basic nitrogens present on the surface of the coated-materials was performed by dipping samples of known surface in 10 mL of 1 M HCl for 1 h. Then the samples were washed with water and dried *in vacuo* (12 h).

The samples were then treated with 0.1 M NaOH for 1 h. Chloride ions in this solution were finally determined by titration with 5×10^{-3} M mercury nitrate solution previously standardized⁵ against KCl.

This titration method is capable to determine only the quantity of nitrogens present on the sample surface. A determination of the basic nitrogens in the deeper layers by a deeper penetration of the titrant¹⁰ was not considered useful here because the different physicochemical characteristics (i.e., swelling, porosity etc.) of the materials blended to PUPA can mislead the titration results.

Heparinization and heparin determination

The heparinization of PUPA-coated polyurethane, PVC, Dacron, and Goretex was obtained by dipping the samples in a 0.5% solution of heparin (sodium salt, from Roche containing 5000 units = 50 mg/mL) in $\text{H}_2\text{O}/\text{C}_2\text{H}_5\text{OH}$ (1:1; v/v) containing 2% of CH_3COOH at 60°C.

Samples of PUPA-coated PVC were also heparinized using the above experimental conditions except with a 0.5% heparin solution in H_2O containing CH_3COOH in the same amount of which above.

The amount of heparin adsorbed on the surface of the PUPA-coated materials was biologically tested by thrombin time (TT) measurements. The samples were stirred in phosphate buffered saline (PBS) solution, to remove all the heparin not bound to the surface. The solution was changed every 60 min until no heparin was monitored by biological test (see below). Secondly the samples were treated with 10 mL of 0.1 M NaOH solution changing it each hour. The solutions were neutralized with HCl solution, and phosphate buffer (pH = 7.4) was added to the solution before determining

the quantity of heparin. The treatment with 0.1 M NaOH solution was repeated till no heparin was monitored by TT measurements.

Thrombin time measurements

The TT test with "Thrombin Reagent" (from Boehringer Mannheim) was performed with an Automatic Elvi 820 Digiclot 2 Coagulimeter (from Logos S.p.A.) A standard curve, obtained by plotting dilutions of heparin (units/mL) versus time, was used to determine the heparin in the eluates.

Obviously, this is a way to determine only the heparin that is still biologically active after the adsorption/desorption process.

RESULTS AND DISCUSSION

By pouring PUPA solution in *N,N'*-dimethylformamide (DMF) on to the surface of the different materials (plasticized polyvinylchloride (PVC), polyurethane (PU), glass, Gore-tex, and Dacron), two different classes of coating were obtained. Due to the solubility of PVC and PU in DMF, the PUPA solution not only covers these surfaces, but also penetrates into the bulk, creating a blend of the two materials. In the case of glass, Gore-tex, and Dacron which are insoluble in DMF, only a layer of PUPA on the surface can be obtained.

The coating of Dacron with PUPA solution first generated a more rigid material due to the thick bridges of PUPA between the Dacron fibers. Hence, PUPA solutions in DMF at different concentrations were used to coat Dacron samples in order to find the right concentration for binding large quantities of heparin without destroying the good mechanical properties of the substrate. Figure 1 shows optical micrographs of Dacron samples coated with 80% (a) and 30% (b) PUPA solutions. The thick bridges between the fibers are not evident in the sample coated with the 30% solution: a thin homogeneous layer of PUPA envelops each Dacron fiber (Fig. 1b), conserving the elasticity of the Dacron material.

FT-IR/ATR spectroscopy

Figure 2 shows the ATR/FT-IR spectra of native and coated PVC, PU, glass, Gore-tex, and Dacron, along with the spectrum of PUPA itself. All the coated-materials spectra reveal the presence of PUPA on the material surface.

Difference spectra and interaction between coating and substrate

PVC and PU. The depth of penetration of PUPA into PVC and PU samples was revealed by slicing the PUPA-coated materials with a microtome and analyzing the layers of known thickness (40 and 50 μm) by FT-IR. Figure 3

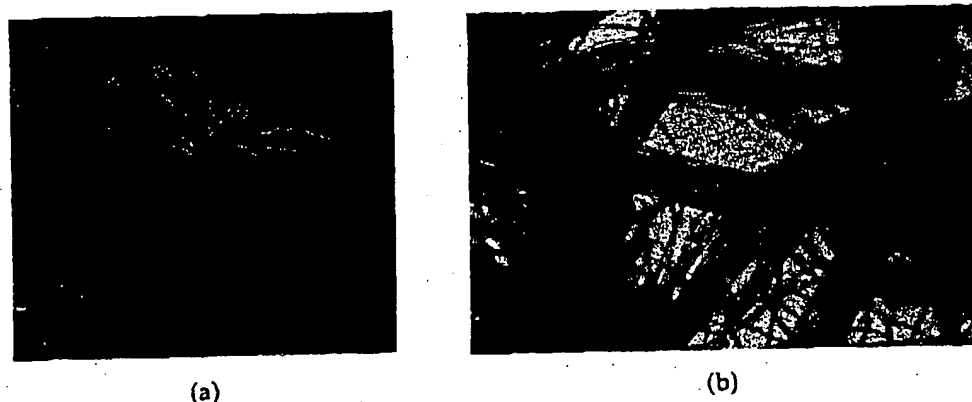


Figure 1. Optical micrographs of Dacron samples coated with: (a) 80% solution of PUPA in DMF, (b) 30% solution of PUPA in DMF.

shows the spectra of these layers for coated PVC and PU samples. In both coated materials, the top layer shows a spectrum very close to that of the native PUPA. The intensity of the PUPA bands decreases, while the intensity of the PVC or PU bands increases with increasing depth, and finally, the layer at about 350 μm shows a spectrum identical to that of the corresponding substrate. This is in accordance with the continuous change in chemical composition of the coated substrate from its surface to its bulk, due to the penetration of PUPA.

(i) *PUPA-coated PVC*: The variations in the PVC spectrum upon coating were analyzed by taking the difference spectrum between PUPA and the coated sample. The difference spectrum is reported in Figure 4 together with that of native plasticized PVC. Comparison of the two spectra shows that the intensity of the plasticizer [dioctylphthalate (DOP)]⁶ bands are higher in the spectrum (a) than in the spectrum (b).

In ATR, the depth of penetration for a nonabsorbing medium, defined as the distance required for the electric field amplitude to fall to e^{-1} of its value at the surface, is given by Harrick⁷:

$$d_p = \frac{\lambda/n_1}{2\pi[\sin^2 \theta - (n_1/n_2)^2]^{1/2}}$$

where n_1 is the refractive index of the sample and n_2 is the refractive index of the ATR element. It has been shown that the sampling depth for polymeric materials is about three times d_p .⁸ If we were analyzing polymeric materials with refractive indices of approximately 1.5, the sampling depth of bands at 1720, 1260, and 960 cm^{-1} would be 3.46, 4.64, and 6.09 μm for a sample analyzed on a 45° KRS-5 element. Considering the penetration of the IR beam, according to this approximation, we can say that the layer investigated was close to the surface. In this layer the different intensity of corresponding peaks suggests an enrichment of DOP within the near-surface sampling depth.

This could mean that the DMF solvent of PUPA preferentially extracted the plasticizer which migrated to the surface interacting with the PUPA-coating material. As a matter of fact, in the ester $\text{C}=\text{O}$ spectral range of the dif-

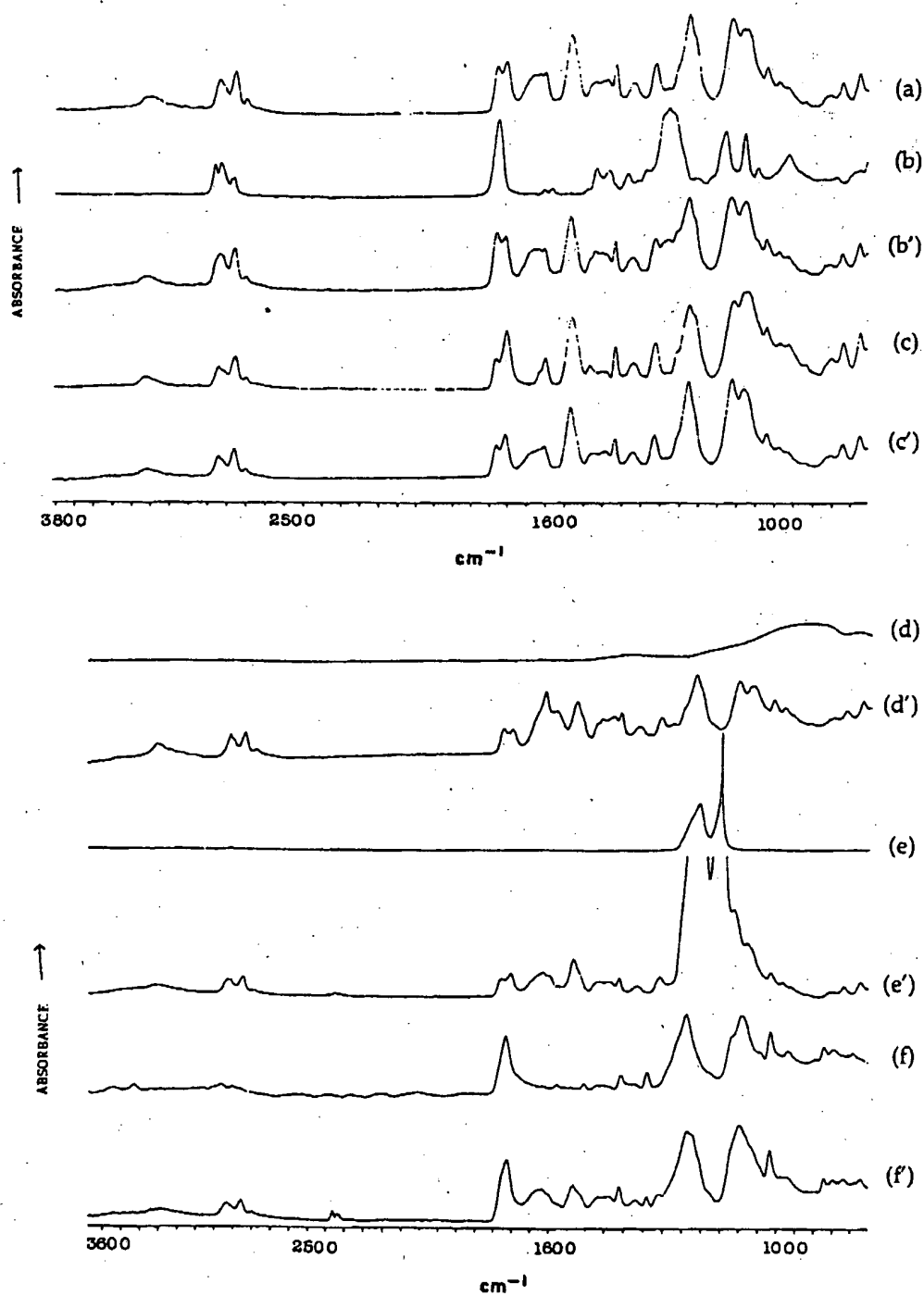


Figure 2. FT-IR/ATR spectra of: (a) PUPA, (b) PVC, (b') PUPA-coated PVC, (c) polyurethane, (c') PUPA-coated polyurethane, (d) glass, (d') PUPA-coated glass, (e) Gore-tex, (e') PUPA-coated Gore-tex, (f) Dacron, (f') PUPA-coated Dacron.

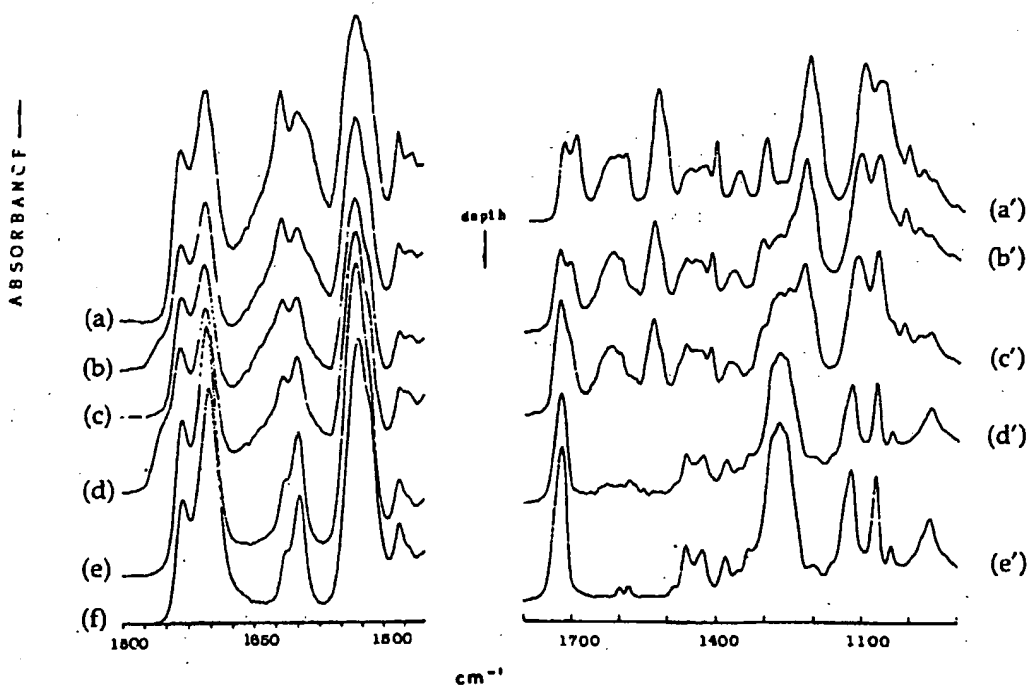


Figure 3. FT-IR/ATR spectra of PUPA-coated polyurethane and PVC. (a) PUPA, (b) top layer, (c) layer 180 μm deep, (d) layer 280 μm deep, (e) layer 380 μm deep of PUPA-coated polyurethane, (f) polyurethane, (a') PUPA, (b') top layer, (c') layer 250 μm deep, (d') layer 350 μm deep of PUPA-coated PVC, (e') PVC.

ference spectrum a shoulder appears at 1706 cm^{-1} ; this shoulder is absent in the spectrum of the native plasticized PVC and can be attributed to a hydrogen-bonding interaction between the ester $\text{C}=\text{O}$ group of DOP and the urethane and/or urea NH group of PUPA. However, this does not mean that separation of the plasticizer from the polyvinylchloride occurred upon coating, but that the coating process only provoked a partial migration of the plasticizer to the surface. The spectrum of the 350- μm deep layer of the coated sample (see Fig. 3) still shows in fact the characteristic bands of plasticizer together with those of polyvinylchloride.

(ii) *PUPA-coated PU*: Since PU is a component of PUPA, in the case of PUPA-coated PU the interactions between coating and underlying material occurred via polyurethane–polyurethane hydrogen bonds.⁹ Subtraction of the substrate spectrum from that of the coated sample also gave information about interaction between the cross-linked poly(amido-amine) and polyurethane chains.¹⁰

The difference spectrum of poly(amido-amine) (Fig. 5a) shows the amide $\text{C}=\text{O}$ stretching at 1623 cm^{-1} whereas this band falls at 1634 cm^{-1} in the free polymer¹⁰ (Fig. 5b). The shift to lower frequency of this band can be attributed to the formation of a hydrogen bond between the amide $\text{C}=\text{O}$ group of poly(amido-amine) and the urethane $\text{N}-\text{H}$ group of polyurethane. As a matter of fact, the difference spectrum also shows a band at 1725 cm^{-1} ,

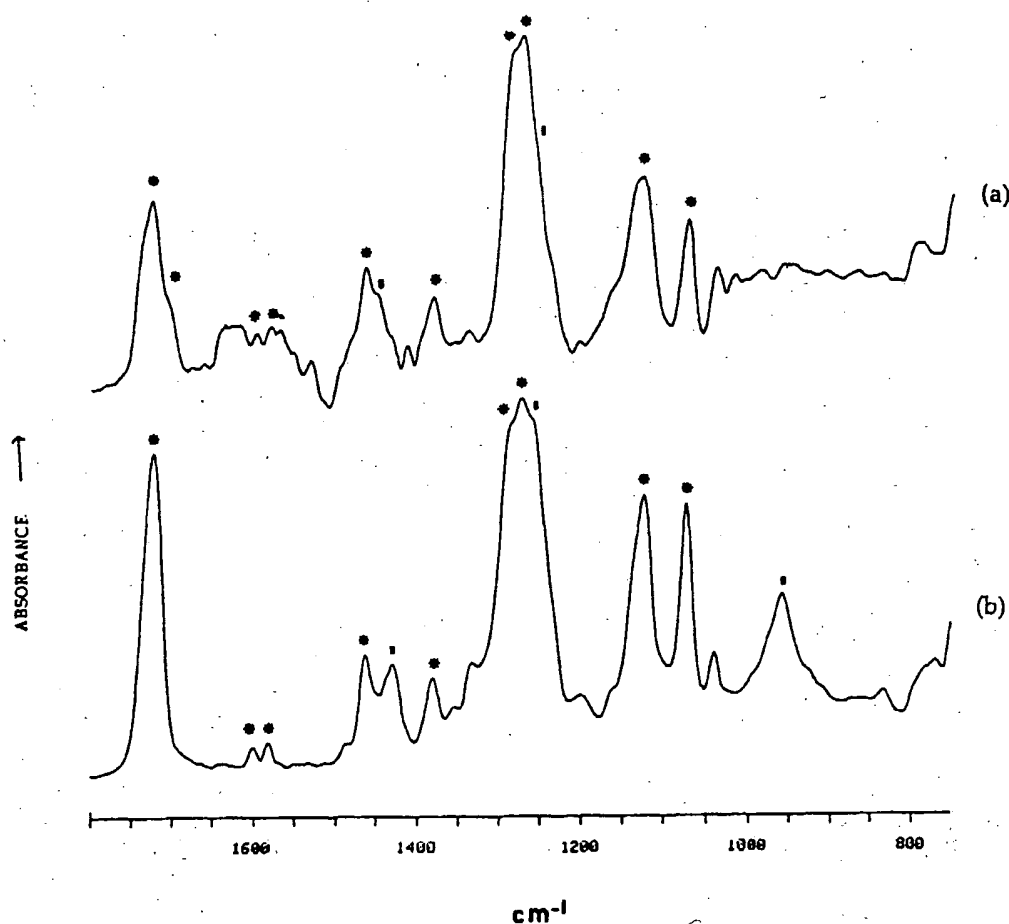


Figure 4. (a) Difference spectrum of PVC obtained from the spectra of PUPA-coated PVC and PUPA, (b) spectrum of native plasticized PVC. * bands due to the DOP, ■ bands due to the PVC.

due to the $C=O$ stretching of the free urethane group.^{10,11} The presence of this band is consistent with the breaking of some urethane-urethane hydrogen bonding in favour of the above urethane-amide interaction.

Glass, Gore-tex, and Dacron

In the case of PUPA-coated glass, Gore-tex, and Dacron, the spectrum of the PUPA coating was obtained by subtracting the spectrum of the substrate from that of the coated sample. Figure 6 shows the difference spectra of PUPA coating on glass (PUPAg), Gore-tex (PUPAG), and Dacron (PUPAD).

In the case of PUPA-coated glass and Dacron, the difference spectrum of PUPA coating does not reveal any particular variation with respect to the spectrum of native PUPA and the difference spectra of glass and Dacron obtained by subtracting the spectrum of PUPA from that of the coated sample were also equivalent to those of the corresponding native substrates. Hence we may assume that only a physical interaction occurs between the coating

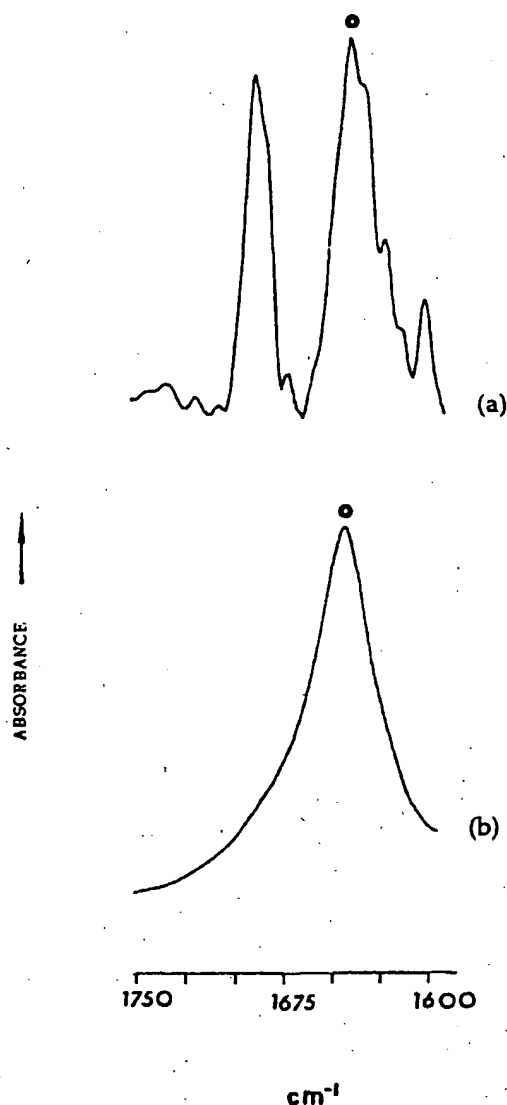


Figure 5. C=O spectral region of: (a) difference spectrum of poly(amido-amine), (b) spectrum of poly(amido-amine) by itself. \bullet band due to the amide C=O stretching.

and the substrate or that the chemical interactions if any, are too weak to be detectable by FT-IR.

In the case of PUPA-coated Gore-tex, however, the difference spectra of PUPA coating and Gore-tex substrate are not the same as those of native PUPA and Gore-tex. In the difference spectrum of PUPA coating (PUPAG) (Fig. 6b), some changes occur in the $1300\text{--}1000\text{-cm}^{-1}$ region with respect to native PUPA. Two bands at 1200 and 1144 cm^{-1} appear as additional positive bands together with the negative peak at 1153 cm^{-1} . Moreover, both the ether C—O—C asymmetric and urethane O=C—O symmetric stretching¹⁰ are shifted to higher frequencies with respect to those in the PUPA spectrum (from 1107 to 1115 cm^{-1} and from 1070 to 1080 cm^{-1} , respectively). This means that in the coated sample some hydrogen bonds between these groups and

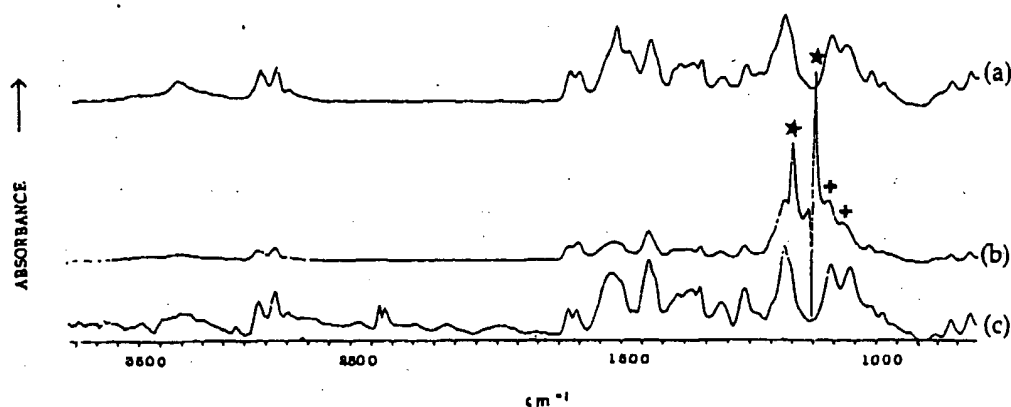


Figure 6. Difference spectra of PUPA-coating on: (a) glass, (b) Gore-tex, (c) Dacron. * bands due to "interacting" C—F group, + bands due to C—O—C asym. and O=C—O sym. stretching of PUPA.

the N—H group, occurring in the PUPA material,⁴ are broken because of the preferential interaction of the latter with the more electronegative C—F group of Gore-tex.

This behavior was revealed by the difference spectrum of Gore-tex obtained by subtracting the spectrum of PUPA from that of the coated sample. In Figure 7, the difference spectrum (a) is compared to the spectrum of native Gore-tex (b) in the 1400–1000-cm⁻¹ range. Comparison of the two spectra shows that the two C—F absorptions at 1210 and 1153 cm⁻¹ of Gore-tex¹² are in part shifted to lower frequencies upon coating. In fact the difference spectrum (a) reveals two strong bands at 1200 and 1144 cm⁻¹ and two shoulders at 1210 and 1153 cm⁻¹. Moreover, the ratio of band intensity obtained by summing the heights of the peak at 1144 and the shoulder at 1153 cm⁻¹ (due to the "interacting"¹³ and "free"¹² C—F stretching, respectively) to that obtained by summing the heights of the peak at 1200 and the shoulder at 1210 cm⁻¹ (due to the "interacting"¹³ and "free"¹² C—F stretching again) in the difference spectrum, is equivalent to the 1153 cm⁻¹/1210 cm⁻¹ intensity peak ratio in the spectrum of native Gore-tex. This emphasizes that some of the C—F groups were involved in a hydrogen-bonding interaction with the coating material. This kind of interaction generally provokes a drop in the absorption frequency of the group involved.¹³

PUPA coating stability investigation

In the case of both coated PVC and PU, a stable coating was formed, due to the penetration feature of PUPA solution in the bulk of the substrate, but in the case of coated glass, Gore-tex, and Dacron, the stability of the PUPA coating was different. In the case of PUPA-coated glass it was found that the layer of coating material can be removed from the substrate after contact with H₂O, DMF, and CH₂Cl₂. The PUPA material swells in these solvents due to the solubility of the poly(amido-amine) in CH₂Cl₂ and H₂O, and of PU in DMF,⁴ allowing the detachment of the PUPA coating from the glass surface by scratching. Other solvents, like acetone or toluene, in which the two components of PUPA are insoluble, did not cause this effect. In the case of Gore-

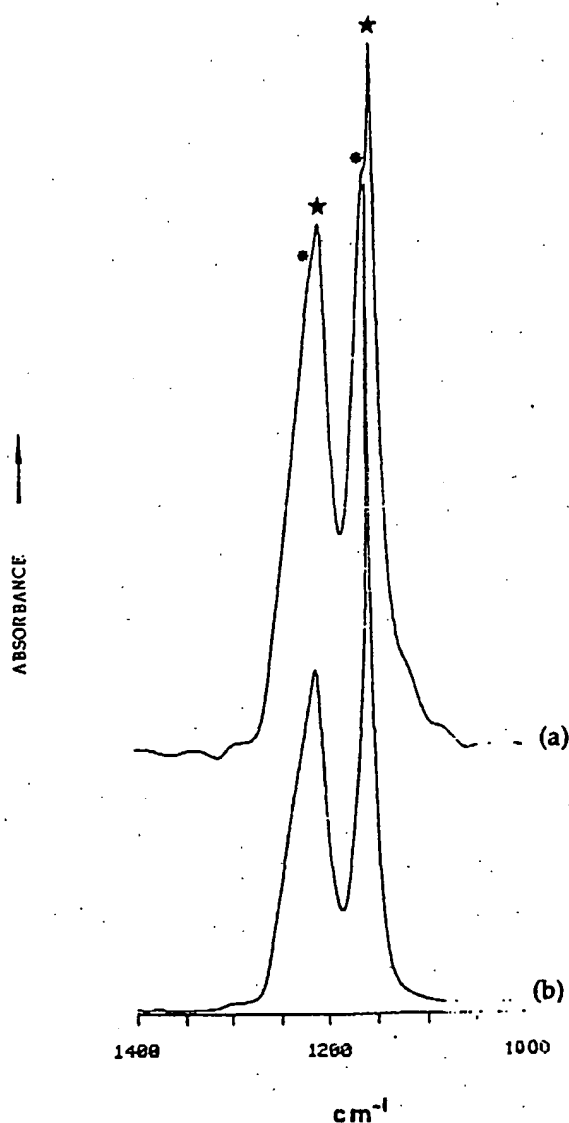


Figure 7. (a) Difference spectrum of Gore-tex obtained from the spectra of PUPA-coated Gore-tex and PUPA, (b) spectrum of native Gore-tex. * bands of native Gore-tex ("free" C—F stretch.), * bands due to "interacting" C—F group.

tex and Dacron, PUPA envelops the fibers and Soxhlet extraction of the coated-samples in $\text{CH}_3\text{OH}/\text{H}_2\text{O}$, was not sufficient to remove the PUPA coating. PUPA coating stability on Gore-tex and Dacron surfaces in flowing saline solution at 37°C revealed that the quantity of PUPA on both sample surfaces remained constant over the period of 1 month, indicating that the layer of PUPA on the substrate is stable at least for this length of time. These results are shown in Table I.

Surface chemical characterization and heparin adsorption

PUPA material has been demonstrated to strongly bind heparin because of the presence of basic aminic nitrogens in its structure. Once protonated,

TABLE I
Stability of PUPA Coating on PVC, PU, Glass, Gore-tex, and Dacron Surfaces under Flowing Saline Solution at 37°C

PUPA-Coated Material	Several days ^c
PVC ^a	∞
PU ^a	∞
Glass	Several days ^c
Gore-tex ^b	≥1 month
Dacron ^b	≥1 month

^aPenetration occurs with blending of the coating and substrate.

^bSamples were weighed before and after exposure to the flowing saline solution. No variation in the sample weight was detected within 1 month.

^cPUPA coating can be removed by scratching.

these may interact with the negatively charged groups of the heparin molecule.⁴⁴ Two kinds of binding occur between heparinized and PUPA surface. The first seems to be physical adsorption, because heparinized PUPA surfaces release heparin when the samples are placed in a stream of saline solution. The second is a stronger ionic bond between the negatively charged groups of heparin and the protonated basic nitrogens of PUPA.¹⁴ This ionically bound heparin is detached from the surface only with 0.1 M NaOH solution, which deprotonates the PUPA N—H⁺ groups.

In the case of PUPA-coated PVC, the heparinization was performed using heparin solution in both H₂O/C₂H₅OH/CH₃COOH and H₂O/CH₃COOH because the DOP plasticizer present in the PVC was partially extracted when the sample was bathed in a solution containing alcohol, generating a very rigid material. This extraction was verified by FT-IR. In the spectrum of the sample soaked in the solution containing alcohol some bands due to the DOP plasticizer decreased in intensity with respect to those of polyvinylchloride.

Using the water/acetic-acid solution the coated material did not change its physicochemical properties. The IR spectrum of the sample soaked in H₂O/CH₃COOH solution was the same as that of the native sample and its flexibility unchanged.

The quantities of the stable heparin on the surface of the different coated materials are summarized in Table II. Because of the impossibility of exactly

TABLE II
Quantity of Titrated Nitrogens and Ionically Bound Heparin on PUPA-Coated Materials

PUPA-Coated Material	Titrated Nitrogens	Ionically Bound Heparin ^a	Hep./Titrated N ^b (mg/μmol)
PVC	1.9 μmol/cm ²	1.0 mg/cm ²	0.5
PU	2.1 μmol/cm ²	1.2 mg/cm ²	0.6
Glass	0.1 μmol/cm ²	0.03 mg/cm ²	0.3
Gore-tex	40.7 μmol/g	4.8 mg/g	0.1
Dacron ^c	50.0 μmol/g	5.7 mg/g	0.1

^aHeparin detached from the surface by 0.1 M NaOH solution.

^bHeparin/titrated nitrogens in PUPA = 0.7 mg/μmol.

^cSurface coated with a 30% solution of PUPA in DMF.

measuring the surface area of PUPA-coated Gore-tex and Dacron, the amount of titrated nitrogens and ionically bound heparin were calculated per unit weight of sample.

The heparin/titrated nitrogens ratios, which are independent from the surface or weight of the considered samples, are reported in Table II.

We can note that a higher ratio is obtained for PUPA-coated PU and PVC than for PUPA-coated Dacron and Gore-tex. That difference can be ascribed to the different thickness of "PUPA layer" which covers the substrates. In the case of PU and PVC we obtain a penetration of PUPA until about 350 μm into the bulk (see Fig. 3). In the case of Dacron and Gore-tex we obtain only a superficial layer of PUPA less than 10 μm thick as evidenced by their IR spectra (see Fig. 2), where the bands of the substrates are present together with those of PUPA.

The heparinization process leads to a full protonation of the poly(amido-amine) chains both on the surface and in the bulk of the material, and the high quantity of heparin bound to PUPA surfaces is explained in terms of the high charge density.^{4,10} In the cases here considered the charge density is of course higher in PUPA-coated PU and PVC than in PUPA-coated Dacron and Gore-tex because of the thickness of "PUPA layer." This justifies the heparin/titrated nitrogens ratio which is higher for the first two samples than for the other ones. As a matter of fact, in the native PUPA sample obtained from the same solution used to coat these substrates, the heparin/titrated nitrogens ratio is about 0.7 (see Table II).¹⁵

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